## *Remarks*

Reconsideration of this Application is respectfully requested.

Upon entry of the foregoing amendment, claims 45-59, and 61-84 are pending in the application, with claims 45, 46, 58, 59 and 72 being the independent claims. Claim 60 is sought to be canceled without prejudice to or disclaimer of the subject matter therein. Claims 72-84 are sought to be added. These changes are believed to introduce no new matter, and their entry is respectfully requested.

Based on the above amendment and the following remarks, Applicants respectfully request that the Examiner reconsider all outstanding rejections and that they be withdrawn.

### *I. Support for Amended and New Claims*

Support for amended claims 45-47 can be found, *inter alia*, in the specification at page 2, line 16, through page 3, line 14.

Support for amended claim 48, 54, 55, 66, and 67 can be found, *inter alia*, in the specification at page 13, line 24, through page 14, line 4.

Support for amended claims 58 and 59 can be found, *inter alia*, in the specification at page 2, line 16, through page 3, line 14; at page 16, line 23, through page 17, line 4; and at page 51, line 6, through page 54, line 14.

Support for new claims 72-80 can be found, *inter alia*, in the specification at page 9, line 15, through page 10, line 14.

Support for new claims 81-84 can be found, *inter alia*, in the specification at page 11, line15, through page 13, line 19.

## *II. Oath/Declaration*

The Examiner acknowledged Applicants' desire to hold the matter of the Oath/Declaration in abeyance. *See* Paper No. 19, page 2. The Examiner, however, stated that the Oath/Declaration remains defective for the reasons set forth in the Office Action mailed on April 12, 1999 (Paper No. 2). Nevertheless, Applicants reiterate their request that the rejection based on the asserted defects with the Oath/Declaration be held in abeyance until the remaining issues outstanding in this application are resolved.

## *III. Obviousness-Type Double Patenting*

Claims 45-71 stand rejected under the doctrine of obviousness-type double patenting. *See* Paper No. 19, page 2. Applicants again respectfully request that this rejection be held in abeyance until the remaining outstanding issues in this application are resolved. As indicated in the Amendment and Reply filed December 5, 2000, once the remaining outstanding issues are resolved, Applicants will file a terminal disclaimer over the '692 patent.

## *IV. Rejections under 35 U.S.C. § 112, First Paragraph*

### *A. Alleged New Matter*

The Examiner has rejected claims 45-57 under 35 USC § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to

reasonably convey to one of ordinary skill in the art that the inventors had possession of the claimed invention at the time the application was filed. *See* Paper No. 19, page 5. More specifically, the Examiner asserted that the amendment to claims 45 and 46, made in Applicants' reply filed December 5, 2000 "represents new matter." *See id.* According to the Examiner, the specification does not support the phrase "wherein said increasing the fatty acid content is not accomplished by growing said bacterium (or bacteria, for claim 46) at a reduced temperature, relative to the temperature at which a bacterium is grown in which the fatty acid content is not increased." *See id.* Applicants respectfully traverse the rejection and contend that the specification provides adequate support for the proviso.

Nonetheless, solely to expedite prosecution, Applicants have amended claims 45 and 46 to remove the above-quoted language. Accordingly, the Examiner's rejection is rendered moot and should therefore be withdrawn.

### *B. Enablement*

The Examiner has rejected claims 45-53, 58-65, 70 and 71 under 35 USC § 112, first paragraph. In particular, the Examiner asserts that

> Applicants' disclosure teaches that increases in unsaturated fatty acids result in increased viability and transformation ability. Applicants' specification is completely silent with respect to what happens with increases in saturated fatty acids or increases in total fatty acids with no change in the ratio between saturated and unsaturated fatty acids.

Paper No. 19, page 4 (emphasis in original). Applicants respectfully traverse the rejection.

Applicants reiterate that the Examiner has not met the initial burden of establishing a *prima facie* case of non-enablement. The Examiner, in explaining the rationale for the

non-enablement rejection, has, in effect, placed the initial burden on Applicants to prove enablement. The burden, however, properly lies in the first instance with the Examiner. *See* MPEP § 2164.04.

Nonetheless, solely to expedite prosecution, Applicants have replaced the recitation of "fatty acid content" in claims 45, 46, 48, 54, 55, 58, 59, 66, and 67 with the phrase "unsaturated fatty acid content." Accordingly, the Examiner's rejection is rendered moot and should therefore be withdrawn.

As another basis for challenging the enablement of Applicants' claims, the Examiner asserted that the specification "fails to enable methods other than the introduction of nucleic acids into the bacteria in order to effect an increase in (unsaturated) fatty acid content." Paper No. 19, page 6. Applicants again respectfully traverse the rejection.

Applicants contend that the specification *does* in fact enable methods of increasing the unsaturated fatty acid content of the membrane of bacteria by means other than introducing nucleic acids into bacteria. For example, the specification in Examples 1 and 16 describes increasing the unsaturated fatty acid content of the membrane of bacteria by *genetic selection*. *See* specification at page16, line 21 - page 17, line 4, and page 51, line 6 - page 54, line 14. More specifically, a technique is described in detail whereby, after four cycles of storing cells at -20°C and selecting for survivors, a strain is isolated possessing an increased unsaturated fatty acid content in its membrane. *See id.* Thus, contrary to the Examiner's assertion, the specification teaches increasing the unsaturated fatty acid content of the membrane of bacteria by methods other than the introduction of nucleic acids.

Notwithstanding the fact that other methods for increasing unsaturated fatty acid content are taught in the specification, Applicants note that they are not limited to the

confines of the specification to provide the necessary information to enable the invention. *See In re* Howarth, 654 F.2d 103, 105-6, 210 USPQ 689, 692 (CCPA 1981). For instance, an applicant need not supply information that is well known in the art. *Howarth*, 654 F.2d at 105-6, 210 USPQ at 692; *see also In re Brebner*, 455 F.2d 1402, 173 USPQ 169 (CCPA 1972) (finding a disclosure enabling because the procedure for making the starting material, although not disclosed, would have been known to one of ordinary skill in the art as evidenced by a Canadian patent). "'That which is common and well known is as if it were written out in the patent and delineated in the drawings.'" *Howarth*, 654 F.2d at 106, 210 USPQ at 692 (quoting *Webster Loom Co. v. Higgins* et al., 105 U.S. (15 Otto.) 580, 586 (1881)). Moreover, one of ordinary skill in the art is deemed to know not only what is considered well known in the art but also where to search for any needed starting materials. *Id.*

At the time the application was filed, multiple methods for increasing the unsaturated fatty acid content of a bacterial membrane were well known in the art. For instance, de Mendoza demonstrated that *E. coli* cells grown at temperatures below 30°C exhibit an enhanced amount of *cis*-vaccenic acid as compared to cells grown at elevated temperatures. *See* de Mendoza *et al.*, *Trends Biol. Sci.* 8:49-52 (1983) at page 49, column 3. Thus, Applicants' claims are clearly enabled, not only by the methods for altering bacterial unsaturated fatty acid content explicitly set forth in the specification, but also by the methods that were known in the art at the time the application was filed. Accordingly, Applicants respectfully request that the rejection under 35 USC § 112, first paragraph, on the basis that the specification fails to teach methods of increasing the fatty acid content of

the membrane of a bacterium/bacteria other than by introducing nucleic acids, be reconsidered and withdrawn.

## *V. Rejections under 35 U.S.C. § 102*

### *A. Inoue et al.*

Claims 58, 59, 63-67, and 71 are rejected under 35 USC § 102(b) as being anticipated by Inoue *et al.*, *Gene* 96: 23-28 (1990) ("Inoue"). *See* Paper No. 19, page 3. Applicants respectfully traverse the rejection.

Claims 58, 59, 63-67, and 71 in their present form are directed to methods for enhancing the transformation ability of a bacterium (claim 58), or of bacteria (claims 59, 63-67, and 71), said methods comprising: (a) increasing the unsaturated fatty acid content of the membrane of said bacteria by (i) enhancing expression of one or more genes that encode one or more gene products which increase said unsaturated fatty acid content, or (ii) genetically selecting for bacteria having an increased membrane unsaturated fatty acid content; and (b) storing said bacteria at a temperature of from about +4°C to about -20°C, wherein said bacteria, after said storing, exhibit enhanced transformation ability relative to the transformation ability exhibited by said bacteria prior to increasing their unsaturated fatty acid content.

Applicants assert that Inoue does not anticipate claims 58, 59, 63-67, and 71 because Inoue does not teach or suggest increasing the unsaturated fatty acid content of the membrane of said bacteria by either (i) enhancing expression of one or more genes that encode one or more gene products which increase said unsaturated fatty acid content, or (ii)

genetically selecting for bacteria having an increased membrane unsaturated fatty acid content. Nor does Inoue teach storing bacteria at a temperature of from about +4°C to about -20°C. Rather, the method taught in Inoue involves the distinct steps of growing bacteria at 18°C followed by storing the bacteria in *liquid nitrogen* before transformation with plasmid DNA. *See e.g.* Inoue at page 24, column 1. No other storage methods or conditions besides liquid nitrogen are taught or suggested.

Because Inoue does not disclose all of the claim limitations of claims 58, 59, 63-67, and 71, Inoue does not anticipate these claims. Accordingly, Applicants respectfully request that the rejection under 35 USC § 102(b) based on Inoue be reconsidered and withdrawn.

***B. de Mendoza et al.***

The Examiner has rejected claims 58-69 and 70 under 35 USC § 102(b) as being anticipated by de Mendoza *et al.*, *J. Biol. Chem.* 258:2098-2101 (1983), and/or de Mendoza *et al.*, *Trends Biol. Sci.* 8:49-52 (1983)[1]. *See* Paper No. 19, pages 3 and 7. Applicants respectfully traverse the rejection.

The de Mendoza paper published in TIBS describes the introduction a *fabB* plasmid into a *fabF1* strain and the resulting synthesis of *cis*-vaccenic acid. *See* de Mendoza (TIBS),

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[1]The rejection is unclear as to which de Mendoza reference the rejection is based on. At page 3 of Paper 19, the Examiner denotes "de Mendoza II" as being the Journal of Biological Chemistry paper that was published in 1983. In the sentence bridging pages 6 and 7, however, the Examiner designates "de Mendoza II" as being the Trends in Biological Sciences paper, also published in 1983. As neither de Mendoza reference anticipates the rejected claims, Applicants' comments presented herein are directed to both the JBC and the TIBS papers. Clarification in the next action is respectfully requested.

at page 51, column 3. This reference does not teach storing bacteria at a temperature of from about +4°C to about -20°C, wherein said bacteria, after said storing, exhibit enhanced transformation ability relative to the transformation ability exhibited by said bacteria prior to the induction of *cis*-vaccenic acid synthesis.

Similarly, in the de Mendoza paper published in JBC, experiments are described wherein, after a plasmid carrying the *fabB* gene is introduced into *fabF*<sup>-</sup> bacteria, crude extracts are prepared from cells grown at 37°C and synthase I activity is measured. *See* de Mendoza (JBC) at page 2099, column 1 and Table I. In another experiment, the fatty acid content of transformed *fabF*<sup>-</sup> cells is analyzed; it is determined that the strains carrying a *fabB* plasmid have "appreciable" quantities of *cis*-vaccenic acid. *See id.* at page 2099, column 2. Besides analyzing enzyme activity and fatty acid content, de Mendoza (JBC) does not teach any other steps.

Moreover, Applicants disagree with the Examiner's conclusion that de Mendoza inherently teaches increased viability *and* increased transformation ability. The Examiner asserted that, in de Mendoza, viability is inherently enhanced for cells in which the copy number of the *fabB* gene is increased. *See* Paper No. 19, page 7. From this assertion, the Examiner then made the unsupported statement that "[t]he viability of a bacterium is the single greatest factor in determining its transformation ability." *See* Paper No. 19, page 8. Based on these two propositions, the Examiner concluded that de Mendoza, by modifying the fatty acid content of the membrane of bacteria, inherently teaches increased viability *and* increased transformation ability.

Applicants contend that the Examiner's reasoning is logically flawed. Even though viability may be *necessary* for efficient transformation ability, it does not follow that

increased viability is *sufficient* to improve transformation ability. The skilled artisan would appreciate that there are many examples of rapidly growing bacterial strains that exhibit poor transformation ability. Therefore, the de Mendoza references do not necessarily inherently teach increasing the viability and transformation ability of bacteria.

Thus, the de Mendoza references do not anticipate claims 58-69 and 70 because neither of these references teach increasing the unsaturated fatty acid content of bacteria and then storing bacteria at a temperature of from about +4°C to about -20°C as recited in amended claims 58 and 59. Nor does de Mendoza necessarily teach a method for enhancing the viability and transformation ability of bacteria. Accordingly, Applicants respectfully request that the rejection under 35 USC § 102(b) based on the de Mendoza reference(s) be reconsidered and withdrawn.

## *Conclusion*

All of the stated grounds of rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding rejections and that they be withdrawn. Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

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## Version with markings to show changes made

45. (Thrice amended) A method for enhancing the transformation ability and the viability of a bacterium, said method comprising:

(a) increasing the unsaturated fatty acid content of the membrane of said bacterium, [wherein said bacterium, after increasing its fatty acid content, exhibits an enhanced transformation ability and enhanced viability after storage] and

(b) storing said bacterium at a temperature of from about +4°C to about -80°C, wherein said bacterium, after said storing, exhibits enhanced transformation ability and enhanced viability relative to the transformation ability and viability exhibited by said bacterium prior to increasing its unsaturated fatty acid content, [, wherein said increasing the fatty acid content is not accomplished by growing said bacterium at a reduced temperature, relative to the temperature at which a bacterium is grown in which the fatty acid content is not increased.]

46. (Thrice amended) A method for enhancing the transformation ability and the viability of bacteria, said method comprising:

(a) increasing the unsaturated fatty acid content of the membrane of said bacteria, [wherein said bacteria, after increasing their fatty acid content, exhibit an enhanced transformation ability and enhanced viability after storage] and

(b) storing said bacteria at a temperature of from about +4°C to about -80°C, wherein said bacteria, after said storing, exhibit enhanced transformation ability and enhanced viability relative to the transformation ability and viability exhibited by said bacteria prior to increasing their unsaturated fatty acid content, [, wherein said increasing the fatty acid content is not accomplished by growing said bacteria at a reduced temperature, relative to the temperature at which cells are grown in which the fatty acid content is not increased.]

47. (Twice amended) The method of claim 46, wherein said [bacteria exhibit said enhanced transformation ability and said enhanced viability after storage] storing of said bacteria is at a temperature of from about +4°C to about -20°C.

48. (Once amended) The method of claim 46, wherein said increasing the unsaturated fatty acid content of the membrane comprises enhancing expression of one or more genes that encode one or more gene products which increase said unsaturated fatty acid content.

54. (Once amended) The method of claim 46, wherein said unsaturated fatty acid is selected from the group consisting of oleic acid, linoleic acid, palmitoleic acid, and cis-vaccenic acid.

55. (Twice amended) The method of claim 54, wherein said unsaturated fatty acid is selected from the group consisting of cis-vaccenic acid and palmitoleic acid.

58. (Twice amended) A method for enhancing the transformation ability of a bacterium, said method comprising:

(a) increasing the unsaturated fatty acid content of the membrane of said bacterium by (i) enhancing expression of one or more genes that encode one or more gene products which increase said unsaturated fatty acid content, or (ii) genetically selecting for a bacterium having an increased membrane unsaturated fatty acid content; [, wherein said bacterium, after increasing its fatty acid content, exhibits an enhanced transformation ability after storage] and

(b) storing said bacterium at a temperature of from about +4°C to about -20°C, wherein said bacterium, after said storing, exhibits enhanced transformation ability relative to the transformation ability exhibited by said bacterium prior to increasing its unsaturated fatty acid content.

59. (Twice amended) A method for enhancing the transformation ability of bacteria, said method comprising:

(a) increasing the unsaturated fatty acid content of the membrane of said bacteria by (i) enhancing expression of one or more genes that encode one or more gene products which increase said unsaturated fatty acid content, or (ii) genetically selecting for a bacteria having an increased membrane unsaturated fatty acid content; [, wherein said bacteria, after increasing their fatty acid content, exhibit an enhanced transformation ability after storage] and

(b) storing said bacteria at a temperature of from about +4°C to about -20°C, wherein said bacteria, after said storing, exhibit enhanced transformation ability relative to the transformation ability exhibited by said bacteria prior to increasing their unsaturated fatty acid content.

61. (Once amended) The method of claim [60] 59, wherein said enhancing expression comprises increasing transcription or translation of said one or more genes.

62. (Twice amended) The method of claim [60] 59, wherein said enhancing expression comprises increasing the copy number of said one or more genes, wherein said one or more genes are comprised by one or more vectors.

66. (Once amended) The method of claim 59, wherein said unsaturated fatty acid is selected from the group consisting of oleic acid, linoleic acid, palmitoleic acid, and cis-vaccenic acid.

67. (Once amended) The method of claim 66, wherein said unsaturated fatty acid is selected from the group consisting of cis-vaccenic acid and palmitoleic acid.

68. (Once amended) The method of claim [60] 59, wherein said one or more genes are selected from the group consisting of a *fabB* gene, a *fabF* gene, a *fabD* gene, a *fabG* gene, a *fabA* gene, a *fabI* gene, a *fabZ* gene, a *fadA* gene, a *fadB* gene, a *fadE* gene, a *fadL* gene, a *fadR* gene, a *farR* gene, and a *fatA* gene.

Claim 60 is cancelled.

New claims 72-84 have been added.